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A multi-centre randomised controlled trial of Transfusion Indication Threshold Reduction on transfusion rates, morbidity and healthcare resource use following cardiac surgery: Study protocol

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ABSTRACT

Thresholds for red blood cell transfusion following cardiac surgery vary by hospital and surgeon. The TITRe2 multi-centre randomised controlled trial aims to randomise 2000 patients from 17 United Kingdom centres, and tests the hypothesis that a restrictive transfusion threshold will reduce postoperative morbidity and health service costs compared to a liberal threshold. Patients consent to take part in the study pre-operatively but are only randomised if their haemoglobin falls below 9 g/dL during their post-operative hospital stay. The primary outcome is a binary composite outcome of any serious infectious or ischaemic event in the first three months after randomisation. Many challenges have been encountered in the set-up and running of the study.

1. Introduction

Over 6% of all red blood cell (RBC) usage in the United Kingdom (UK) occurs in cardiac surgery [1]. RBC transfusion is essential in some cardiac surgical patients for the management of life-threatening haemorrhage. In most cases, however, decisions to give a RBC transfusion are made because the Hb concentration has fallen to a level or threshold at which the physician is uncomfortable [2,3]. The transfusion threshold varies across different cardiac surgery units and between different surgeons, which contributes to the wide variation in blood usage observed in cardiac surgical units (25–95%) [4]. The threshold variation stems from a lack of evidence regarding what

Abbreviations: AE, adverse event; AKI, acute kidney injury; CRF, case report form; CABG, coronary artery bypass graft; DMEC, Data Monitoring and Ethics Committee; GP, general practitioner; Hct, Haematocrit; Hb, Haemoglobin; ICU, intensive care units; ICH GCP, International Conference for Harmonisation of Good Clinical Practice; MI, myocardial infarction; NHS, National Health Service; NICE, National Institute for Health and Care Excellence; QALYs, quality adjusted life years; RBC, red blood cell; RCT, randomised controlled trial; REC, Research Ethics Committee; RRT, renal replacement therapy; SAE, serious adverse event; SIRS, systemic inflammatory response syndrome; TRICC, Transfusion Requirements in Critical Care; UK, United Kingdom.

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constitutes a safe level of anaemia following cardiac surgery.

Viral, bacterial or prion infection, and haemolytic transfusion reactions are well publicised risks of RBC transfusion, but these are rare [5]. Immunosuppression, lung injury or organ dysfunction, on the other hand, may potentially occur in every recipient [6]. The risk of pneumonia, estimated to be approximately 13% following coronary artery bypass graft (CABG) surgery, has been observed to increase by 5% per unit of RBCs or platelets transfused [7]. In addition, retrospective studies investigating associations between RBC transfusion and specific morbidity after cardiac surgery have shown associations with nosocomial pneumonia [8], sternal wound infection [9], and severe sepsis [10]. A comparison of propensity matched pairs of transfused versus non-transfused patients, using data from over 3000 patients treated in 145 European ICUs, observed that RBC transfusion conferred a relative risk of mortality of 1.4 (95% confidence interval 1.24–1.36) [11]. RBC transfusion has also been reported to be associated with an increase in mortality up to five years after cardiac surgery [2,12].

In addition to the direct costs of blood products, RBC transfusion may increase hospital costs by prolonging ICU and hospital stay. In abdominal [13] and orthopaedic surgery [14], avoiding RBC transfusion was associated with a reduction in total treatment costs of approximately £7000 per patient in 2012 prices. Findings of increased mortality up to five years after surgery suggest that there may be costs arising from long term transfusion-related morbidity or delayed complications [2,12]. There are also wider resource issues relating to the use of blood components nationally. Donor blood is an increasingly scarce resource, with up to 10% of donors excluded as a consequence of variant Creutzfeldt–Jakob disease restrictions on the donor pool [5]. Increasing scarcity and the costs associated with attracting new donors, as well as the introduction of additional measures aimed at increasing the safety of donated blood, are very likely to increase the direct costs of RBC transfusion.

There is little evidence about the optimal transfusion threshold for cardiac surgery patients. Healthy human subjects can tolerate Hb levels as low as 5 g/dL without adverse consequences [15], and Hb levels as low as 7 g/dL are safely tolerated in non-cardiac surgery, trauma, and ICU patients [16]. The FOCUS trial hypothesised that elderly trauma patients undergoing hip surgery randomised to a liberal (Hb level <10.0 g/dL) transfusion trigger would recover faster than those randomised to a restrictive (Hb level <8.0 g/dL) trigger [17]. The trial demonstrated no superiority for the liberal transfusion trigger and the authors concluded that a more restrictive threshold should be used, because of the absence of harm and the clear economic benefit of using less blood. In contrast, the Canadian Transfusion Requirements in Critical Care (TRICC) study hypothesised that non-cardiac ICU patients randomised to a restrictive (Hb level <7.0 g/dL) would do no worse than those randomised to a liberal (Hb level <10.0 g/dL) transfusion trigger [18]. The restrictive trigger resulted in a 54% relative reduction in RBC transfusion and also a reduction in the frequency of organ dysfunction and 30-day mortality, effects which were attributed to a reduction in red cell transfusion associated morbidity. A subsequent meta-analysis of TRICC and other studies confirmed that reducing RBC transfusion thresholds reduced postoperative transfusion rates, further supporting the use of more restrictive thresholds. Cardiac complications showed a non-significant reduction [19].

However, the applicability of these observations to a cardiac surgery population is unclear because the level of anaemia considered to be 'safe' is thought to be higher in the presence of cardiac disease. A post hoc analysis of the subgroup of patients with coronary artery disease in the TRICC study found no difference in 30-day mortality between the restrictive and liberal threshold groups [20]. On the basis of the TRICC study results, some cardiac units in the UK routinely use a transfusion trigger of 7 g/dL without any apparent detriment to patients [21,22]. However, to date, there has been no high quality randomised trial of different post-operative RBC transfusion thresholds in a UK population of cardiac surgery patients. The most recent randomised controlled trial (RCT) of liberal versus restrictive transfusion thresholds in cardiac surgery patients randomised 502 patients in a hospital in Brazil. No difference was found between the groups (testing a hypothesis of non-inferiority) but the study randomised all patients consented before surgery and was underpowered for a clinically important non-inferiority margin [23].

1.1. Aims and objectives

We have undertaken a multi-centre RCT of Transfusion Indication Threshold Reduction (TITRe2) on transfusion rates, morbidity and healthcare resource use following cardiac surgery. The trial is designed to test the hypothesis that a restrictive threshold for RBC transfusion (Hb 7.5 g/dL and/or haematocrit (Hct) 22%) will reduce post-operative morbidity and health service costs compared to a liberal threshold (Hb 9 g/dL and/or Hct 27%).

Specific objectives of this multi-centre RCT are to:

A. Estimate the difference in the risk of a post-operative infection or ischaemic event between restrictive and liberal transfusion thresholds.
B. Compare the effects of restrictive and liberal transfusion thresholds with respect to a range of secondary outcomes.
C. Estimate the cost-effectiveness of a restrictive compared to a liberal Hb transfusion threshold.

2. Patients and methods

2.1. Trial design

The study is a multi-centre RCT comparing two alternative transfusion thresholds, restrictive versus liberal thresholds, for RBC transfusion in the UK National Health Service (NHS).[1]

[1] Trial registration: The trial was registered as ISRCTN70923932 before starting to recruit.

2.2. Participants

Seventeen UK cardiac surgery centres are participating in the study. Participant eligibility criteria are as inclusive as possible to promote the applicability of the evidence obtained during the trial; see Table 1 for inclusion and exclusion criteria.

Potential trial participants receive a patient information leaflet describing the study, either in the post or faxed to the hospital where they are waiting for surgery, and are then seen in hospital by a member of the research team who answers any questions, confirms eligibility and obtains written informed consent before surgery. Details of reason(s) for non-participation (e.g. reason for being ineligible or refusal) are carefully documented wherever possible. For patients who consent, post-operative Hb and/or Hct levels from blood samples analysed as part of the patient's usual care are monitored carefully; if the Hb level drops below 9.0 g/dL or Hct below 27% then the registered patient becomes eligible for randomisation. Randomisation should take place as soon as possible after the Hb level has dropped below 9.0 g/dL or Hct below 27%, and at the latest within 24 h. Patients are eligible for randomisation at any time during their post-operative stay, irrespective of whether: (a) a RBC transfusion has been given prior to randomisation; (b) a prior breach of the 9.0 g/dL Hb/Hct of 27% threshold was missed; or c) any element of the primary outcome has occurred. See Fig. 1 for an illustration of the trial schema.

The duration of intervention in the trial is the duration of the patient's care under the consultant cardiac surgeon or a maximum of three months after the date of randomisation, whichever is shorter. Almost always, the duration of care under the cardiac surgeon is the period of hospitalisation after surgery. The duration of follow-up in the trial is until three month follow-up assessment questionnaires have been completed or until three months after randomisation if a participant refuses to complete the questionnaires.

2.3. Interventions

The two groups are defined as follows:

Liberal (control, similar to current practice): Transfuse if post-operative Hb level falls below 9.0 g/dL or Hct falls below 27%.

Restrictive (experimental): Transfuse if post-operative Hb level falls below 7.5 g/dL or Hct falls below 22%.

Thresholds can be expressed either as Hb or Hct because anaemia is assessed by varying instruments across centres and stages of care (intensive care and ward). Where both Hb and Hct values are available, transfusion is indicated if either of these values falls below the allocated threshold (7.5 g/dL or Hct of 22% versus 9.0 g/dL or Hct of 27%). Hereafter, only Hb levels are referred to but this should be interpreted as Hb or Hct.

The objective is to maintain the Hb level at or above 9.0 g/dL in the liberal group and at or above 7.5 g/dL in the restrictive group. For both groups Hb levels are monitored and transfusions given accordingly for the duration of the patient's post-operative hospital stay on the cardiac ICU or cardiac surgical ward. If the Hb drops below ("breaches") the allocated threshold a RBC transfusion should be given as soon as possible, at least within 24 hours. One unit of RBC should be transfused at a time and the Hb level checked before transfusing another unit. Clinicians are allowed to transfuse, or refuse to transfuse, in contravention of the allocated threshold but the reason(s) why must be documented on the study case report form (CRF). Other aspects of post-operative care are provided in accordance with usual care.

2.4. Outcomes

2.4.1. Primary outcome

The primary outcome is a binary composite outcome of any serious infectious (sepsis or wound infection) or ischaemic event (stroke, MI, gut infarction or AKI) in the first three months after randomisation. Qualifying events and the manner in which they are verified are detailed in Table 2.

Events occurring post-discharge only contribute to the primary outcome if the potentially qualifying event results in admission to hospital or death, except for post-discharge wound infections; these are ascertained using a modified ASEPSIS post-discharge wound assessment (see Table 2) [24,25]. Documentary evidence about events suspected to qualify for the primary outcome is sent to the co-ordinating centre and verified by research nurses who are blinded to allocation. Suspected post-randomisation MIs are

Table 1
Inclusion/exclusion criteria.

Inclusion criteria:
- Adults of either sex, aged 16 years or over undergoing cardiac surgery (defined as CABG, valvular or aortic surgery or surgical correction of congenital cardiac disease)
- Post-operative Hb level below 9.0 g/dL or Hct below 27 at any stage during the patient's post-operative hospital stay
- Written informed consent

Exclusion criteria:
- Patients undergoing emergency cardiac surgery (defined as surgery taking place before the end of the same working day as admission)
- Patients who are prevented from having blood and blood products according to a system of beliefs (e.g. Jehovah's Witnesses)
- Patients with congenital or acquired platelet, red cell or clotting disorders (patients with iron deficient anaemia are not excluded)
- Patients with ongoing or recurrent sepsis
- Patients unable to give full informed consent for the study (e.g. learning or language difficulties)
- Patients with critical limb ischaemia (defined as rest pain in affected limb associated with peripheral vascular disease)
- Patients already participating in another interventional research study

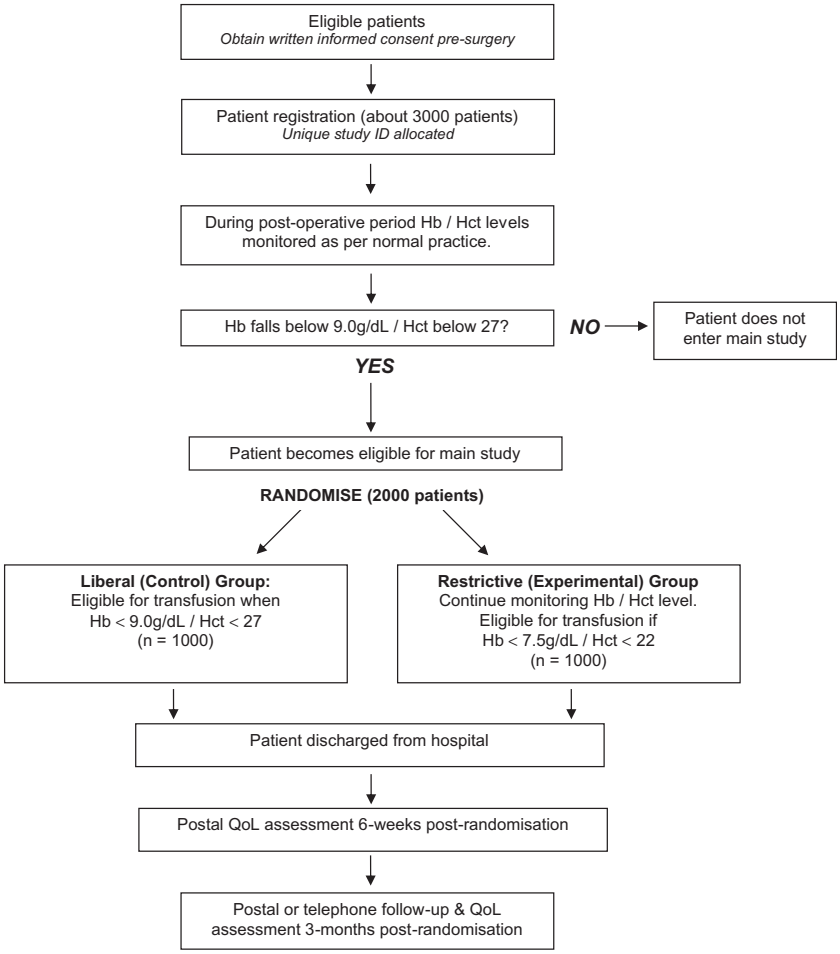


Fig. 1. Trial schema.

adjudicated by an independent committee comprising a cardiac surgeon, anaesthetist and cardiologist who are external to the study and blinded to allocation. Death is not included as a component of the primary composite outcome because, if death occurs because of one of the included components, the component will precede death itself. Deaths that occur for other reasons are not hypothesised to increase because of RBC transfusion.

2.4.2. Secondary outcomes

The following secondary outcomes are collected in the three month follow-up period (unless otherwise stated):

- Units of RBCs and other blood components transfused during a participant's hospital stay.
- Proportion of patients experiencing an infectious event.
- Proportion of patients experiencing an ischaemic event.
- Health-related quality of life using the EuroQol EQ5D [26].
- Duration of ICU / high dependency unit post-operative stay;
- Duration of post-operative hospital stay.
- All-cause mortality.
- Cumulative resource use, cost, and cost-effectiveness.
- Significant pulmonary morbidity, comprising: (a) initiation of non-invasive ventilation (e.g. continuous positive airway pressure ventilation); (b) re-intubation/ventilation; and/or (c) tracheostomy. This outcome was added after starting recruitment as a proxy for transfusion related acute lung injury.

In addition, all serious adverse events (SAEs) in the 3 month follow-up period are reported (both expected events listed in the study protocol and unexpected events).

2.5. Data collection

For all participants who consent, data collection pre-operatively consists of medical history, demographic data, Hb level, medication use and type of cardiac surgery (e.g CABG, valve implantation). The participant is also asked to complete a generic quality of life questionnaire, the EuroQol EQ5D questionnaire. The lowest Hb recorded on each post-operative day is documented.

For randomised participants all transfusions are recorded, including any transfusion decisions that are

Table 2
Definition of serious infectious/ischaemic events for primary outcome.

	Definition/method of verification
Infectious events	
Sepsis during index admission	Defined by the following two conditions, both of which must be satisfied for sepsis to be documented: (a) Antibiotic treatment for suspected infection, *and* (b) The presence of systemic inflammatory response syndrome (SIRS)[a] within 24 h prior to start of antibiotic treatment
Wound infection	ASEPSIS [38] score >20. Wounds will be assessed at least once during a participant's hospital stay and details of the ASEPSIS assessment added to the study CRF. A questionnaire will be posted for self-completion, or will be administered by telephone, at 3 months to identify wound infections arising after discharge [24]
Ischaemic events	
Permanent stroke	Clinical report of brain imaging (computed tomography or magnetic resonance imaging), in association with new onset focal or generalised neurological deficit (defined as deficit in motor, sensory or co-ordination functions)
MI	Elevated post-operative peak serum Troponin I or T[b]
AKI	AKI Network criteria for AKI, stage 1, 2 or 3 (see below) [39]
	Stage 1: Serum creatinine increase $\geqslant$0.3 mg/dl ($\geqslant$26.4 μmol/l), OR >1.5 and $\leqslant$2-fold serum creatinine increase compared to the pre-operative serum creatinine (baseline) value, OR Urine output <0.5 ml/kg for 6 h.
	Stage 2: >2 and $\leqslant$3-fold serum creatinine increase compared to the pre-operative serum creatinine (baseline) value, OR Urine output 0.5 ml/kg for >12 h
	Stage 3: >3-fold serum creatinine increase compared to the pre-operative serum creatinine (baseline) value, OR Serum creatinine $\geqslant$4.0 mg/dl ($\geqslant$354 μmol/l) with an acute increase of at least 0.5 mg/dl (44 μmol/l), OR Urine output <0.3 ml/kg per hour for 24 h or anuria for 12 h, OR Need for renal replacement therapy (RRT) irrespective of AKI stage at time of RRT
Gut infarction	Laparotomy or post mortem

[a] SIRS is central to the diagnosis of infective complications. It will be defined as $\geqslant$2 of the following conditions: temperature >38 °C or <36 °C; heart rate >90 beats/minute; respiratory rate >20 breaths/min or $PaCO_2$ <32 mm Hg or <4.3 kPa; white blood cell count >12,000/mm^3 or <4000/mm^3. Blood test results and temperature will be classified using standard reference ranges.
[b] Criterion levels of troponin I and T for defining a post-operative MI have not been established. MIs will be adjudicated by an independent committee blinded to allocation.

inconsistent with the allocated threshold. For such decisions (i.e. either a transfusion is given when the threshold is not breached, or a transfusion withheld when the threshold is breached) the Hb level at the time and reason for making the decision are documented. During a participant's hospital stay, data are collected for the primary outcome (e.g. temperature, heart rate, respiratory rate, results of haematology and biochemistry investigations, ASEPSIS assessments of wounds for infection), secondary outcomes (e.g. duration of intensive/high dependency care), and other key resources used (e.g. return to theatre, medications, units of blood components transfused).

The consultant responsible for a participant may decide it is in the best interests of a participant to permanently discontinue treatment according to the allocated group. If so, then the reason(s) for this decision must be documented. The participant is not withdrawn from the trial and the participant is followed up. If a participant withdraws consent at any time, the participant no longer forms part of the study but may be included in the analysis cohort if willing for data already collected to be used.

Three months after randomisation, a questionnaire is posted for self-completion or administered by telephone. It includes the following elements: (a) adverse events (AEs) occurring after discharge, with further details of any event suspected to contribute to the primary outcome or to meet the definition of a SAE being sought, e.g. from the admitting hospital or the participant's general practitioner (GP); (b) questions to identify surgical wound infections occurring after discharge (ASEPSIS post-discharge surveillance questionnaire) [24]; (c) resource use questionnaire; (d) questions determining whether a participant is aware of his/her random allocation.

Finally, the EuroQol EQ5D [26] is posted to participants at six weeks and three months post-randomisation. Participants who are registered into the study but are not randomised also receive the EuroQol EQ5D three months after their operation. Descriptive summaries of their EQ5D scores will be compared to those of randomised patients.

2.6. Sample size

The trial is designed to test a superiority hypothesis. The following steps were undertaken to calculate the required sample size:

1. Risks of transfusion in the two groups are critical to the success of the trial. Data for the distribution of nadir Hb and Hct from an observational analysis published shortly before funding was awarded are shown in Fig. 2 [2]. Based on these data, we assumed that approximately 65% of patients would breach the threshold of 9.0 g/dL, and approximately 20% would breach the 7.5 g/dL threshold.

2. Therefore, with complete adherence to the transfusion protocol, we assumed that 100% of participants randomised to the liberal group and 30% of participants randomised to the restrictive group (≈0.20/0.65) would be transfused.
3. In the observational analysis [2], 63% of patients with a nadir Hct between 22.5% and 27% and 93% of patients with a nadir Hct below 22.5 were transfused. Therefore, in combination with the proportions of patients expected to breach the liberal and restrictive thresholds, these figures were used to give conservative estimated transfusion rates of 74% for the liberal group and ⩽35% for the restrictive group, i.e. assuming some non-adherence with the transfusion protocol severe enough to alter transfusion rates in each group (Fig. 3).
4. The observational estimate of the relative risk for any compared to no transfusion was adjusted to reflect the estimated transfusion rates in the two groups and combined with the frequency of infections and ischaemic events observed in the untransfused group, giving event rates of the proposed composite outcome of 17% in the liberal threshold group, and 11% in the restrictive threshold group, a risk difference of 6%. A sample size of 1468 was required to detect this difference with 90% power with 5% significance (2-sided test).
5. The target sample size was inflated to 2000 patients (i.e. 1000 in each arm) to allow for uncertainty about non-adherence that affects transfusion rates and the estimated proportions of patients experiencing the primary outcome. We regarded these parameter estimates as uncertain because: (a) they were estimated from observational data; (b) they were based on the RBC transfusion rate only in Bristol for over seven years; (c) they were based on routinely collected data, using definitions for elements of the composite primary outcome which are not identical to those proposed for the trial; (d) they were based on any versus no RBC transfusion, rather than on the number of units of RBCs likely to be transfused in patients who breach the liberal threshold.

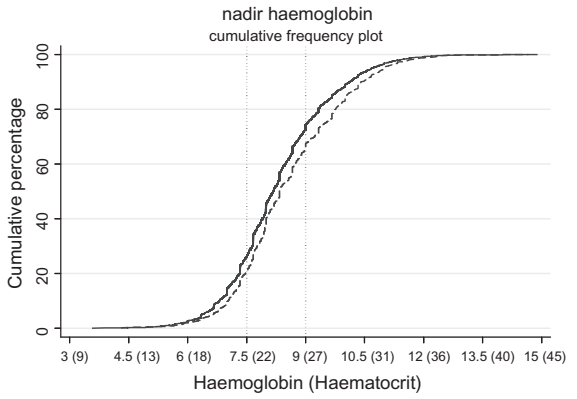


Fig. 2. Data for the distribution of nadir haemoglobin/haematocrit from recent observational analysis [2] both for the entire dataset ($n = 8621$, solid line) and for the most recent data (2003; $n = 1106$, dashed line). Vertical lines represent the restrictive and liberal protocols compared in this trial. Notes: The most recent data show a slight shift in the cumulative frequency plot towards higher Hb, probably because of wider uptake of off-pump CABG surgery, which causes less blood loss, over the period represented by the data. Data describing nadir Hb were obtained specifically for the analysis and we have not linked the clinical and haematology databases for more recent years. However, the proportion of offpump CABG has not continued to increase since 2003.

The primary outcome measure for the economic evaluation is quality adjusted life years (QALYs). The analysis will include baseline QALYs as a covariate and the correlation between baseline and three month assessments of QALYs was assumed to be ⩾0.3. With a total sample size of 2000, the trial will have >95% power to detect a standardised difference in continuous outcomes between groups of 0.2% with 1% significance (2-sided test). This magnitude of difference is conventionally considered to be "small" [27].

2.7. Randomisation

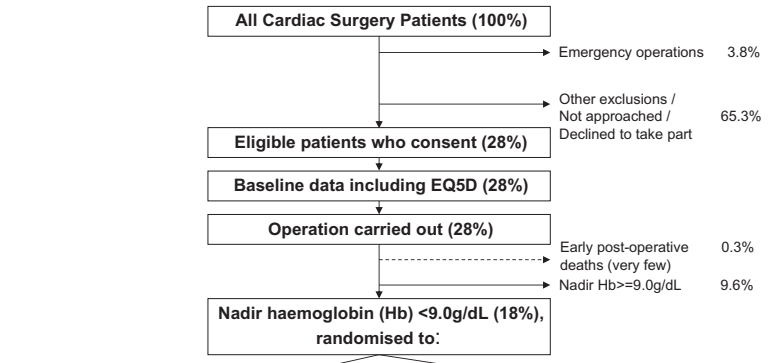
Participants are randomised using a third party internet-based system (Sealed Envelope Ltd.; www.sealedenvelope.com). Staff in participating centres access the system using a password and pin number. Patients become eligible for randomisation if their haemoglobin falls below 9 g/dL at any point post-operatively. At this time information to identify a participant uniquely and to confirm eligibility must be entered before the system assigns a randomisation number and the treatment allocation, ensuring concealment of allocations. Participants are allocated to the liberal or restrictive transfusion strategies in a 1:1 ratio, and cohort minimisation is used to achieve balance across the two arms of the trial; minimisation factors are centre and operation type (CABG only; Valve only; CABG and valve; Other).

2.8. Blinding

Every effort is made to blind participants to their allocation. The success of participant blinding is checked by asking participants if they knew what their allocation was at the time of their discharge from hospital and at three months. It is not possible to blind clinicians and other health care staff caring for patients to the random allocation of participants. Therefore, special care has been taken to define outcomes on the basis of objective criteria, or adjudication, in order to reduce the risk of bias.

2.9. Statistical methods

All analyses of primary and secondary outcomes will be on an intention-to-treat basis. Full details of the statistical methods used are given elsewhere [28]. In brief, the primary outcome will be analysed using logistic regression with the following pre-specified subgroup analyses being carried out: (a) operation type, (b) age, (c) pre-operative diagnosis of diabetes or not, (d) pre-operative diagnosis of lung disease, (e) pre-operative renal impairment, (f) sex and (g) ventricular function. Secondary outcomes will be analysed using logistic regression, Cox regression or linear regression, as appropriate. An interim analysis will be carried out after 50% of patients have been recruited and



	TRANSFUSION THRESHOLD		
	Nadir Hb <7.5g/dL	Nadir Hb ≥7.5 to <9.0 g/dL	Total
TRANSFUSED	96%	62%	74%
NOT TRANSFUSED	4%	38%	26%

	TRANSFUSION THRESHOLD		
	Nadir Hb <7.5g/dL	Nadir Hb ≥7.5 to <9.0 g/dL	Total
TRANSFUSED	96%	<5%	<35%
NOT TRANSFUSED	4%	>=95%	>=65%

Fig. 3. CONSORT diagram summarising TITRe 2 trial design. Notes: Percentages are based on data from the cardiac surgery registry in Bristol for the period Jan to Sep 2007. An unknown percentage of patients are excluded by the exclusion criteria because the registry does not contain sufficient detail to apply the definitions proposed for the trial. However, patients meeting one or more of these criteria are extremely rare and we expect all of the exclusion criteria to account for a maximum of 5% of cardiac surgery patients.

followed for 3 months. Ancillary, observational analyses will be carried out: (a) estimating the dose-response relationship between the number of RBC units transfused and the risk of mortality and morbidity, with the findings of these analyses compared for consistency with previous findings [2]; (b) investigating whether RBC 'age' is associated with the risk of both primary and secondary outcomes; and (c) investigating of the relationship between the percentage decline in Hb (from preoperative value) and the risk of primary and secondary outcomes.

2.10. Economic evaluation

The economic evaluation is being undertaken from an NHS and personal social services perspective. A cost-utility analysis is being conducted since the primary outcome measure for the economic evaluation is QALYs. Established guidelines from National Institute for Health and Care Excellence (NICE) (UK) will be followed [29].

Resource use data collection has been integrated into the study CRFs. During the index hospital admission, detailed data are being collected on blood products transfused, inpatient days by ward type, surgery and re-operations, medications and any complications and their treatment. At three months post-randomisation, a bespoke resource use questionnaire is used to obtain estimates of healthcare resources used since hospital discharge, for example readmissions to hospital and further contact with health professionals in primary or secondary care such as outpatient appointments and GP visits. Unit costs from nationally published sources such as NHS Reference Costs and Unit Costs of Health and Social Care 2012 [30,31] will then be applied to these resources, and the total costs per patient calculated.

QALYs will be estimated using the EuroQol EQ5D, which is administered to patients pre-operatively, and at 6 weeks and 3 months post-randomisation [26]. Respondents will be assigned valuations derived from published UK population tariffs [32] and the QALYs gained per patient calculated.

Any missing cost and outcome data will be dealt with using multiple imputation methods [33] The average costs and QALYs gained in each trial arm will be calculated, and from this the incremental cost-effectiveness ratio will be derived, producing an incremental cost per QALY gained of the restrictive threshold compared to the liberal threshold. The restrictive threshold will be considered cost-effective if the incremental cost-effectiveness ratio falls below £20,000, the level below which NICE generally recommends interventions to the NHS [34].

Deterministic and probabilistic sensitivity analysis will be used to assess the impact on results of variation around key parameters such as costs for treatments of complications. Results will be expressed in terms of a cost-effectiveness acceptability curve, which indicates the likelihood that the restrictive threshold is cost-effective for different levels of willingness to pay for health gain.

2.11. Safety reporting

Safety data are collected for all participants for the duration of their follow-up in the trial. AEs and SAEs are defined as required by International Conference for Harmonisation of Good Clinical Practice (ICH GCP). Many AEs, including death, are listed in the protocol as 'expected occurrences not subject to expedited reporting' (see Table 3 for a list of such events). Other AEs that meet the definition of a SAE are defined as unexpected SAEs. Furthermore, an

Table 3
Expected adverse events listed in the study protocol.

Any element of the infectious/ischaemic events as part of the composite primary outcome, including:
• Sepsis
• Wound infection
• Permanent stroke
• MI
• AKI
• Gut infarction
Transient ischaemic attack
Other gastro-intestinal complications, including:
• Pancreatitis
• Obstruction or perforation
Post-operative haemorrhage
Cardiac tamponade
Pulmonary complications, including:
• Acute respiratory distress syndrome
• Re-intubation and ventilation
• Tracheostomy
• Initiation of mask continuous positive airway pressure ventilation after weaning from ventilation
• Pneumothorax requiring chest drainage
• Pleural effusion requiring drainage
Arrhythmias, including:
• Supraventricular tachycardia or atrial fibrillation requiring treatment
• Ventricular fibrillation or tachycardia requiring intervention
• Pacing
Re-operation for any reason, including:
• Bleeding
• Cardiac arrest
• Mediastinitis
Thromboembolic complications, including:
• Deep vein thrombosis
• Pulmonary embolus
Low cardiac output, requiring management with a Swan-Ganz catheter, an intra-aortic balloon pump, or left ventricular assist device
Wound dehiscence requiring rewiring or treatment for reason other than infection
Death

unexpected related SAE is defined as an unexpected SAE that is judged by the local Principal Investigator to be possibly, probably or definitely related to allocation to one or other arm of the trial.

SAEs are recorded and reported in accordance with the ICH GCP guidelines and the Sponsor's Research Related Adverse Event Reporting Policy. Data on all AEs and SAEs are collated and reported regularly to the Data Monitoring and Ethics Committee (DMEC), distinguishing SAEs that occur in the same participants.

2.12. Funding and regulatory bodies

The trial is funded by the National Institute for Health Research Health Technology Assessment Programme (Project Number 06/402/94) and sponsored by University Hospitals Bristol Foundation NHS Trust. The study protocol has been reviewed and approved by the 'Oxfordshire Research Ethics Committee (REC) C' (REC ref: 08/H0606/125). The study is being conducted in accordance with: relevant aspects of the Medicine for Human Use (Clinical Trial) Regulations 2004; ICH GCP guidelines; Research Governance Framework for Health and Social Care; the UK Data Protection Act 1998.

3. Discussion

The TITRe2 trial presents a unique opportunity to answer a fundamental question about a clinical intervention that is administered to 90% of cardiac surgery patients in some centres and over 50% of all patients in the UK and elsewhere. When the trial was conceived, there was no previous high quality RCT of alternative RBC transfusion thresholds in patients having cardiac surgery to date (other than the pilot study for this trial, which was greatly underpowered). It is important to investigate this question in patients having cardiac surgery because, compared to other patient populations, these patients are considered to be at greater risk of myocardial ischaemia due to coronary artery disease, and systemic tissue hypoxia in the presence of severe anaemia due to impaired cardiac output. In addition, cardiac surgery is a specialty which uses a large amount of blood. Increasing recognition of the risks of RBC transfusion [35], coupled with the increasing costs of this potentially diminishing resource, had led to calls for good quality RCTs to determine the relative risks and benefits of anaemia and RBC transfusion in this population [36]. However, there have been several obstacles to overcome in setting up and conducting the study.

The choice of Hb thresholds for transfusion proved problematic. Thresholds for transfusion are controversial and different people argue for different thresholds. Our choice of thresholds takes into account that: (a) the thresholds span a 'densely populated' part of the distribution of nadir Hb (see Fig. 2); (b) in cardiac intensive care, either transfusing at Hb >9.0 g/dL or not transfusing until the Hb drops below 7.0 g/dL was considered unacceptable by most clinicians; (c) a reasonable difference between the

liberal and restrictive thresholds was needed for a trial with a feasible sample size. Therefore, the thresholds represent a compromise which spans the range of contemporary international practice. Among clinicians in interested centres, despite some unease at transfusing outside their existing protocols in some instances, there is a willingness to accept the proposed thresholds because of the perceived urgency of addressing the research question.

Assumptions for the sample size calculation highlighted our concern about adherence. The DMEC was, in particular, anxious that transfusions in the liberal group might be withheld or delayed causing convergence of the transfusion rates of the two groups, particularly as interested sites tended to err on the lower side of transfusion thresholds in cardiac surgery. Therefore, complex methods of monitoring and categorising non-adherence have been implemented. Two main types of non-adherence have been identified: (a) giving transfusions outside of protocol and (b) withholding transfusions, and both types have been further categorised into mild, moderate or severe depending on the likely impact on transfusion risks (any transfusion versus no transfusion). We believe that TITRe2 is unique in attempting to document the latter.

Severe instances are those that will affect the overall transfusion rate in each group. Mild and moderate can only affect the total numbers of RBC units and/or timing of transfusions. Currently around 38% of patients have had one or more instances of non-adherence with the transfusion protocol, with the instance deemed "severe" for approximately 8% of patients. The former figure is similar to the overall non-adherence rate of 38% from a recent pilot study investigating adherence to transfusion strategies in cardiac surgery; however, in this pilot study the intervention period included the intra-operative period [37]. The latter figure of approximately 8% "severe" non-adherence is comparable to the frequency of severe protocol breaches reported in the recent FOCUS trial [17]. Whilst rates of non-adherence in TITRe2 are somewhat high, these rates have been deemed acceptable by the DMEC, reflecting the pragmatic nature of the study. Furthermore, the severe non-adherence rate, which impacts on the comparison between groups of the proportion having any transfusion, is consistent with the assumptions underpinning the sample size calculation.

At the start of the trial, recruitment was much slower than anticipated due to delays in finalising and obtaining signed site agreements and NHS approvals. Once sites started recruiting, the initial projection of 19 consented patients per site per month was found to be over-optimistic (although achieved by the Chief Investigator's site), with sites achieving eight consented patients per month on average. Slower recruitment is thought to be due to a number of reasons including: (a) staff availability for the study in local site teams, with the impact of research nurse annual leave and sick leave extending to one week either side of actual absence; (b) emerging competing studies at some sites not registered at the TITRe2 set-up stage; (c) not all surgeons at sites being willing to take part. The study management team examines recruitment rates and characteristics of sites frequently and holds discussions with sites about rate-limiting steps to recruitment. Newsletters and regular investigator meetings (with research nurses attending) are also used to update sites and to reinforce important messages. Finally, the randomisation proportion has been lower than the projected 65%, meaning that more patients need to be consented into the study than anticipated. At the start of the study the randomisation proportion was less than 50%; however examination of the characteristics of randomised and non-randomised patients led to recommendations to sites to target patients having off-pump cardiac surgery, as well as urgent (inpatient) cases, where possible. This has led to an increase in the randomisation proportion to around 58%, although this is still somewhat lower than originally predicted. Some eligible patients have also been missed, contributing to the reduced randomisation proportion. All of these issues led to a successful application to the funder to extend the duration of the trial and open additional centres. During the course of the extension recruitment has been consistent with the revised projections.

Data collection is complex and more time-consuming than anticipated. In order to ensure that transfusions are given according to the protocol for the allocated group, Hb levels are required for every post-operative day, as well as at each "breach" of the allocated threshold and at each transfusion. The timing of each breach and transfusion is also required, along with the reasons why a transfusion is not given when indicated, or given outside of protocol. Although most patients are discharged within 10 days of their operation, around 25% of the study population stay longer (so far the longest stay has been 85 days) and data collection for these patients is demanding. In addition, the need to use objective criteria to define primary outcome events (e.g. sepsis) means data collection is multifaceted with a number of different observations required. In response to feedback from sites the study CRFs were substantially revised after approximately nine months of recruitment, with consequent improvement in the efficiency of data collection. In addition, the payment per patient that sites receive has been increased to reflect the higher than predicted number of hours of research nurse time required for each patient.

The primary outcome event rate has been substantially higher than anticipated in the sample size calculation (approximately 34%). This appears to be due to: (a) on average, a higher-risk population being randomised into the study than in the previous observational study, and (b) the use of objective criteria to define sepsis and AKI, compared to clinical recording of these events. Consequently, a sensitivity analysis focusing only on more serious events has been included in the statistical analysis plan [28].

4. Conclusion

In summary, despite the complex nature of the study and challenging data collection requirements, the trial has proceeded successfully. Lessons learnt from TITRe2 should help to design and conduct future transfusion based studies.

Authors' contributions

RCMB: study design, preparation and drafting of study protocol, review of manuscript.

KP: central monitoring including adherence, statistical analysis plan, drafting of manuscript.

AM: study design, preparation and drafting of study protocol.

SW: study design, preparation and drafting of study protocol, design of health economic component, review of manuscript.

EAS: drafting of health economic component of manuscript.

ADM: study design, preparation of study protocol.

AC: study design, preparation of study protocol.

GDA: study design, preparation of study protocol, review of manuscript.

GJM: study concept, study design, preparation and drafting of study protocol, review of manuscript. Lead clinical investigator of the trial.

CAR: study design, preparation and drafting of study protocol, sample size and statistical analysis plan, review of manuscript.

BCR: study concept, study design, preparation and drafting of study protocol, drafting and review of manuscript. Chief investigator of the trial.

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References

[1] Wells AW, Mounter PJ, Chapman CE, Stainsby D, Wallis JP. Where does blood go? Prospective observational study of red cell transfusion in north England. BMJ 2002;325:803.
[2] Murphy GJ, Reeves BC, Rogers CA, et al. Increased mortality, postoperative morbidity and cost following red blood cell transfusion in patients having cardiac surgery. Circulation 2007;116:2544–52. Epub 2007 November 12.
[3] Corwin HL, Parsonnet KC, Gettinger A. RBC transfusion in the ICU: is there a reason? Chest 1995;108:767–71.
[4] Stover EP, Siegel LC, Parks R, Levin J, Body SC, Maddi R, et al. Variability in transfusion practice for coronary artery bypass surgery persists despite national consensus guidelines: a 24-institution study. Institutions of the multicenter study of perioperative ischemia research group. Anesthesiology 1998;88:327–33.
[5] Regan F, Taylor C. Blood transfusion medicine. BMJ 2002;325:143–7.
[6] Spahn DR, Casutt M. Eliminating blood transfusions: new aspects and perspectives. Anesthesiol 2000;93:242–55.
[7] Vamvakas EC, Carven JH. Transfusion and postoperative pneumonia in coronary artery bypass surgery: effect of the length of storage of transfused red cells. Transfusion 2000;40:832–9.
[8] Leal-Noval SR, Marquez-Vácaro JA, Garcia-Curiel A, et al. Nosocomial pneumonia in patients undergoing heart surgery. Crit Care Med 2000;28:935–40.
[9] Zacharias A, Habib RH. Factors predisposing to median sternotomy complications. Deep vs superficial infection. Chest 1996;110:1173–8.
[10] Michalopoulos A, Stavridis G, Geroulanos S. Severe sepsis in cardiac surgical patients. Eur J Surg 1998;164:217–22.
[11] Vincent JL, Baron J-F, Reinhart K, et al. Anemia and blood transfusion in critically ill patients. JAMA 2002;288:1499–507.
[12] Engoren MC, Habib RH, Zacharias A, Schwann TA, Riordan CJ, Durham SJ. Effect of blood transfusion on long-term survival after cardiac operation. Ann Thorac Surg 2002;74:1180–6.
[13] Jensen LS, Grunnet N, Hanberg Sorensen F, Jorgensen J. Cost-effectiveness of blood transfusion and white cell reduction in elective colorectal surgery. Transfusion 1995;35:719–22.
[14] Blumberg N, Kirkley SA, Heal JM. A cost analysis of autologous and allogeneic transfusions in hip-replacement surgery. Am J Surg 1996;171:324–30.
[15] Weiskopf RB, Viele MK, Feiner J, et al. Human cardiovascular and metabolic responseto acute, severe isovolemic anemia. JAMA 1998;279:217–21.
[16] Carson JL, Hill S, Carless P, Hébert PC, Henry D. Transfusion triggers: a systematic review of the literature. Trans Med Rev 2002;16:187–99.
[17] Carson JL, Terrin ML, Noveck H, Sanders DW, Chaitman BR, Rhoads GG, et al. Liberal or restrictive transfusion in high-risk patients after hip surgery. N Engl J Med 2011;365:2453–62.
[18] Hebert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion requirements in critical care investigators, canadian critical care trials group. N Engl J Med 1999;340:409–17.
[19] Hébert PC, Yetisir E, Martin C, et al. Is a low transfusion threshold safe in critically ill patients with cardiovascular diseases? Crit Care Med. 2001;29:227–34.
[20] Hill SR, Carless PA, Henry DA, et al. Transfusion thresholds and other strategies for guiding allogeneic red blood cell transfusion. Cochrane Database Syst Rev 2000(1). http://dx.doi.org/10.1002/14651858.CD00204.
[21] Murphy GJ, Allen SM, Unsworth-White J, Lewis CT, Dalrymple-Hay MJR. Laboratory coagulation and lytic parameters resulting from autotransfusion of washed salvaged red cells following coronary artery bypass grafting: a randomised controlled trial. Ann Thorac Surg 2004;77:1553–9.
[22] Murphy GJ, Modi P, Allen SM, Unsworth-White J, Lewis CT, Dalrymple- Hay MJR. Economic outcome of perioperative cell salvage and autotransfusion following coronary artery bypass grafting: a randomised trial. Presented to the European Society of Cardiothoracic Surgeons September, 2004.
[23] Hajjar LA, Vincent J-L, Galas FRBG, et al. Transfusion requirements after cardiac surgery – the TRACS randomized controlled trial. JAMA 2010;304:1559–67.
[24] Wilson AP, Weavill C, Burridge J, Kelsey MC. The use of the wound scoring method 'ASEPSIS' in postoperative wound surveillance. J Hosp Infect 1990;16:297–309.
[25] Gibbons C, Bruce J, Carpenter J, Wilson AP, Wilson J, Pearson A, et al. Identification of risk factors by systematic review and development of risk-adjusted models for surgical site infection. Health Technol Assess 2011;15(30):1–156. iii–iv.
[26] EuroQol Group. EuroQol – a new facility for the measurement of health-related quality of life. Health Policy 1990;16:119–208.
[27] Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. New Jersey: Lawrence Erlbaum; 1988. p. 24–7.
[28] Pike K, Nash RL, Murphy GJ, Reeves BC, Rogers CA. Update on the Transfusion Indication Threshold Reduction (TITRe2) trial in cardiac surgery: statistical analysis plan. Submitted for publication.
[29] National Institute for Health and Care Excellence (NICE). A Guide to the Methods of Technology Appraisal. London, NICE, 2013. http://publications.nice.org.uk/guide-to-the-methods-of-technology-appraisal-2013.
[30] Department of Health. NHS Reference Costs 2011–12. London: Department of Health; 2012.
[31] Curtis L. Unit costs of health and social care 2012. Canterbury: The University of Kent; 2012.
[32] Kind P, Dolan P, Gudex C, Williams A. Variations in population health status: results from a United Kingdom national questionnaire survey. BMJ 1998;316:736–41.
[33] Briggs A, Clark T, Wolstenholme J, Clarke P. Missing presumed at random: cost-analysis of incomplete data. Health Econ 2003;12(5):377–92.

[34] National Institute for Health and Clinical Excellence. Social value judgements: Principles for the development of NICE guidance, In: 2nd ed., 2008. http://www.nice.org.uk/media/C18/30/SVJ2PUBLICATION2008.pdf.
[35] Koch CG, Li L, Duncan AI, Mihaljevic T, Cosgrove DM, Loop FD, et al. Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. Crit Care Med 2006;34:1608–16.
[36] Walsh TS, McClelland DBL. When should we transfuse critically ill and perioperative patients with known coronary artery disease. Br J Anaesth 2002;89:719–22.
[37] Shehata N, Burns LA, Nathan H, Hebert P, Hare GMT, Fergusson D, et al. A randomized controlled pilot study of adherence to transfusion strategies in cardiac surgery. Transfusion 2012;52:91–9.
[38] Wilson AP, Treasure T, Sturridge MF, Gruneberg RN. A scoring method (ASEPSIS) for postoperative wound infections for use in clinical trials of antibiotic prophylaxis. Lancet 1986;1:311–3.
[39] Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. Acute kidney injury network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 2007;11:R31.